

## SUMMARY

### **The Effect Of Cholecalciferol On GLUT4 Proteins Translocation Of Skeletal Muscle Cells In Streptozotocin-induced Hyperglycemia Mice**

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Diabetes mellitus (DM) is a global health burden because the ever-increasing incidence, high mortality and morbidity and the economic impact in the treatment and prevention efforts is significant. DM patients have chronic hyperglycemia due to relative insulin deficiency and insulin resistance in target organs (muscle cells and adipose cells). Glucose uptake into skeletal muscle cells requires insulin-dependent and insulin-independent signaling pathways, both leading to the translocation of glucose transporter-4 (GLUT4) to the plasma membrane. Insulin resistance occurs due to failure of insulin signaling to translocate GLUT4 from intracellular membranes to the muscle cells, resulting in the failure of glucose uptake and causing hyperglycemia. Increase of muscle GLUT4 content has become a potential pharmacological target to ameliorate hyperglycemia. DM treatment includes lifestyle modification, diet, exercise, oral antidiabetic drugs (metformin, sulfonylurea, thiazolidinedione) and insulin. Efficacy of OAD was well received, but have not been able to restore insulin sensitivity and have not been able to prevent degeneration of pancreatic beta cells. Long-term use of OAD can also cause side effects and tolerance. The development of antihyperglycemia drugs now shifted to the activation of molecules that can enhance GLUT4 translocation into the muscle cells and adipose cells so as to increase insulin sensitivity, one of them are 5'-AMP-activated kinase (AMPK) activation via increasing cytosolic  $\text{Ca}^{2+}$ .

Cholecalciferol is one form of vitamin D3. Vitamin D3 is known to have a function in regulating calcium homeostasis was shown to increase the synthesis of insulin by beta cells of the pancreas, increasing insulin sensitivity in peripheral and it had been proven that the decreased levels of vitamin D3 is associated with increased risk of cardiovascular complications in patients with DM.

Cholecalciferol can increase GLUT4 translocation through an alternative pathway that is independent of insulin is to increase calcium ( $\text{Ca}^{2+}$ ) cytosol.

The purpose of this study is to explain the role of cholecalciferol to increased insulin sensitivity in hyperglycemia mice models through increased GLUT4 translocation in skeletal muscle cells.

This study is a laboratory experimental research with the study design randomized post -test only control group design, using simple random sampling. 30 mice that meet the inclusion and exclusion criteria adapted for one week and then induced using single dose 150mg/kgBW streptozotocin(STZ) intraperitoneally, hyperglycemia occurred on the third day with an average fasting blood sugar levels  $374.57 \pm 73.90$  mg /dL. After experiencing hyperglycemia mice were divided into 5 groups, namely the negative control group (STZ+propylene glycol), the first group (STZ+25ng cholecalciferol), group II (STZ+50ng cholecalciferol), group III (STZ+100ng cholecalciferol), and the positive control group (STZ+metformin ). Cholecalciferol given orally for 14<sup>th</sup> days. On day 15<sup>th</sup> the examination of fasting blood sugar levels were taken and the mice were sacrificed so then the gastrocnemius muscle tissue can be taken for immunohistochemical examination. Fasting blood sugar levels measured using a glucometer, Immunohistochemical examination using rat GLUT4 polyclonal antibodies. The assesment of protein GLUT4 was conducted by quantifying ImmunoReactive Score (IRS-GLUT4).

The analysis used in this study is the analysis one way ANOVA with an error rate of 5%, and regression analysis by using SPSS 17 programe in order to get the correlations between the doses response relationship. Based on statistical analysis showed that there were significant differences in fasting blood sugar levels between treatment groups ( $p < 0,001$ ) also found significant differences in the number of cells of skeletal muscle GLUT4 translocation between treatment groups ( $p < 0,001$ ). Univariate regression analyses showed that cholecalciferol was positively correlated with expression GLUT4 protein in skeletal muscle ( $p < 0,001$ ) and negative correlation of cholecalciferol with fasting blood glucose ( $p < 0,001$ ).

Mechanism cholecalciferol alleged role in lowering blood glucose levels through a direct effect on insulin secretion choecalciferol. Cholecalciferol is

inactivated by the enzyme 1- $\alpha$ -hydroxylase in pancreatic beta cells to its active form is 1,25(OH)<sub>2</sub>D<sub>3</sub> which binds to its receptor in pancreatic beta cells. Further 1,25(OH)<sub>2</sub>D<sub>3</sub> binds to vitamin D response element ( VDRE ) in the insulin gene promoter that activates transcription of the insulin gene so that the end result is increased insulin synthesis. Insulin secretion is a Ca<sup>2+</sup>-dependent process .Cholecalciferol to maintain the balance of intracellular Ca<sup>2+</sup> is thus able to increase insulin secretion. Cholecalciferol also have receptors on skeletal muscle cells, cholecalciferol binding to its receptor produces genomic effects and non-genomic effects, which in turn will keep the balance of cytosolic Ca<sup>2+</sup> to increase the stimulation of GLUT4 translocation to the cell membrane. From this study it can be concluded that cholecalciferol may lower fasting blood sugar levels and increase protein GLUT4 translocation in skeletal muscle cell membranes in hyperglycemia mice models.

